Section II

Biological Risk Assessment

Risk assessment is an important responsibility for directors and principal investigators of microbiological and biomedical laboratories. Institutional biosafety committees (IBC), animal care and use committees, biological safety professionals, and laboratory animal veterinarians share in this responsibility. Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs.

Laboratory directors and principal investigators should use risk assessment to alert their staffs to the hazards of working with infectious agents and to the need for developing proficiency in the use of selected safe practices and containment equipment. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building, and the public.

Risk assessment requires careful judgment. Adverse consequences are more likely to occur if the risks are underestimated. By contrast, imposition of safeguards more rigorous than actually needed may result in additional expense and burden for the laboratory, with little safety enhancement. Unnecessary burden may result in circumvention of required safeguards. However, where there is insufficient information to make a clear determination of risk, it is prudent to consider the need for additional safeguards until more data are available.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. In addition, the capability of the laboratory staff to control hazards must be considered. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.

The agent summary statements contained in BMBL identify the primary agent and procedure hazards for specific pathogens and recommend precautions for their control. The guest editors and contributors of this and previous editions of BMBL based their recommendations on an assessment of the risks associated with the handling of pathogens using generally routine generic laboratory procedures. A review of the summary statement for a specific pathogen is a helpful starting point for assessment of the risks of working with that agent and those for a similar agent.

HAZARDOUS CHARACTERISTICS OF AN AGENT

The principal hazardous characteristics of an agent are: its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease. The

World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on these principal characteristics and the route of transmission of the natural disease.[1] The four groups address the risk to both the laboratory worker and the community. The *NIH Guidelines* established a comparable classification and assigned human etiological agents into four risk groups on the basis of hazard.[2] The descriptions of the WHO and NIH risk group classifications are presented in Table 1. They correlate with but do not equate to biosafety levels. A risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level. See Chapter 3 for a further discussion of the differences and relatedness of risk groups and biosafety levels.

TABLE 1
CLASSIFICATION OF INFECTIOUS MICROORGANISMS BY RISK GROUP

RISK GROUP CLASSIFICATION	NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES 2002[2]	WORLD HEALTH ORGANIZATION LABORATORY BIOSAFETY MANUAL 3RD EDITION 2004[1]
Risk Group 1	Agents that are not associated with disease in healthy adult humans.	(No or low individual and community risk) A microorganism that is unlikely to cause human or animal disease.
Risk Group 2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk).	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk).	(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.[3]

Other hazardous characteristics of an agent include probable routes of transmission of laboratory infection, infective dose, stability in the environment, host range, and its endemic nature. In addition, reports of LAIs are a clear indicator of hazard and often are sources of

information helpful for identifying agent and procedural hazards, and the precautions for their control. The absence of a report does not indicate minimal risk. Reports seldom provide incidence data, making comparative judgments on risks among agents difficult. The number of infections reported for a single agent may be an indication of the frequency of use as well as risk. Nevertheless, reporting of LAIs by laboratory directors in the scientific and medical literature is encouraged. Reviews of such reports and analyses of LAIs identified through extensive surveys are a valuable resource for risk assessment and reinforcement of the biosafety principles. The summary statements in BMBL include specific references to reports on LAIs.

The predominant probable routes of transmission in the laboratory are: 1) direct skin, eye or mucosal membrane exposure to an agent; 2) parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors; 3) ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and 4) inhalation of infectious aerosols. An awareness of the routes of transmission for the natural human disease is helpful in identifying probable routes of transmission in the laboratory and the potential for any risk to the public health. For example, transmission of infectious agents can occur by direct contact with discharges from respiratory mucous membranes of infected persons, which would be a clear indication that a laboratory worker is at risk of infection from mucosal membrane exposure to droplets generated while handling that agent. The American Public Health Association publication *Control of Communicable Diseases Manual* is an excellent reference for identifying both natural and often noted laboratory modes of transmission.[3] However, it is important to remember that the nature and severity of disease caused by a laboratory infection and the probable laboratory route of transmission of the infectious agent may differ from the route of transmission and severity associated with the naturally-acquired disease.[4]

An agent capable of transmitting disease through respiratory exposure to infectious aerosols is a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. This hazard requires special caution because infectious aerosols may not be a recognized route of transmission for the natural disease. Infective dose and agent stability are particularly important in establishing the risk of airborne transmission of disease. For example, the reports of multiple infections in laboratories associated with the use of *Coxiella burnetii* are explained by its low inhalation infective dose, which is estimated to be ten inhaled infectious particles, and its resistance to environmental stresses that enables the agent to survive outside of a living host or culture media long enough to become an aerosol hazard.[5]

When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents require careful consideration in risk assessment. Evidence that experimental animals can shed zoonotic agents and other infectious agents under study in saliva, urine, or feces is an important indicator of hazard. The death of a primate center laboratory worker from Cercopithecine herpesvirus 1 (CHV-1, also known as monkey B virus) infection following an ocular splash exposure to biologic material from a rhesus macaque emphasizes the seriousness of this hazard.[6] Lack of awareness for this potential hazard can make laboratory staff vulnerable to an unexpected outbreak involving multiple infections.[7] Experiments that demonstrate transmission of disease from an infected animal to a normal animal housed in the same cage are reliable indicators of hazard. Experiments that do not demonstrate transmission, however, do not rule out hazard. For example, experimental animals infected with *Francisella tularensis*,

Coxiella burnetii, *Coccidioides immitis*, or *Chlamydia psittaci*, agents that have caused many LAIs, rarely infect cagemates.[8]

The origin of the agent is also important in risk assessment. Non-indigenous agents are of special concern because of their potential to introduce risk of transmission, or spread of human and animal or infectious diseases from foreign countries into the United States. Importation of etiological agents of human disease requires a permit from the CDC. Importation of many etiological agents of livestock, poultry and other animal diseases requires a permit from the USDA's Animal and Plant Health Inspection Service (APHIS). For additional details see Appendix F.

Genetically-modified agent hazards. The identification and assessment of hazardous characteristics of genetically modified agents involve consideration of the same factors used in risk assessment of the wild-type organism. It is particularly important to address the possibility that the genetic modification could increase an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments. The risk assessment can be difficult or incomplete, because important information may not be available for a newly engineered agent. Several investigators have reported that they observed unanticipated enhanced virulence in recent studies with engineered agents.[9-12] These observations give reason to remain alert to the possibility that experimental alteration of virulence genes may lead to increased risk. It also suggests that risk assessment is a continuing process that requires updating as research progresses.

The *NIH Guidelines* are the key reference in assessing risk and establishing an appropriate biosafety level for work involving recombinant DNA molecules.[2] The purpose of the *NIH Guidelines* is to promote the safe conduct of research involving recombinant DNA. The guidelines specify appropriate practices and procedures for research involving constructing and handling both recombinant DNA molecules and organisms and viruses that contain recombinant DNA. They define recombinant DNA as a molecule constructed outside of a living cell with the capability to replicate in a living cell. The *NIH Guidelines* explicitly address experiments that involve introduction of recombinant DNA into Risk Groups 2, 3, and 4 agents, and experiments in which the DNA from Risk Groups 2, 3, and 4 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. Compliance with the *NIH Guidelines* is mandatory for investigators conducting recombinant DNA research funded by the NIH or performed at, or sponsored by, any public or private entity that receives any NIH funding for recombinant DNA research. Many other institutions have adopted these guidelines as the best current practice.

The *NIH Guidelines* were first published in 1976 and are revised on an ongoing basis in response to scientific and policy developments. They outline the roles and responsibilities of various entities affiliated with recombinant DNA research, including institutions, investigators, and the NIH. Recombinant DNA research subject to the *NIH Guidelines* may require: 1) approval by the NIH Director, review by the NIH Recombinant DNA Advisory Committee (RAC), and approval by the IBC; or 2) review by the NIH Office of Biotechnology Activities (OBA) and approval by the IBC; or 3) review by the RAC and approvals by the IBC and Institutional Review Board; or 4) approval by the IBC prior to initiation of the research; or 5) notification of the IBC simultaneous with initiation of the work. It is important to note that review by an IBC is required for all non-exempt experiments as defined by the *NIH Guidelines*.

The *NIH Guidelines* were the first documents to formulate the concept of an IBC as the responsible entity for biosafety issues stemming from recombinant DNA research. The NIH Guidelines outlines the membership, procedures, and functions of an IBC. The institution is ultimately responsible for the effectiveness of the IBC, and may define additional roles and responsibilities for the IBC apart from those specified in the NIH Guidelines. See Appendix J for more information about the *NIH Guidelines* and OBA.

Cell cultures. Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. This risk is well understood and illustrated by the reactivation of herpes viruses from latency,[13,14] the inadvertent transmission of disease to organ recipients,[15,16] and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) within infected individuals in the U.S. population.[17] There also is evidence of accidental transplantation of human tumor cells to healthy recipients which indicates that these cells are potentially hazardous to laboratory workers who handle them.[18] In addition, human and animal cell lines that are not well characterized or are obtained from secondary sources may introduce an infectious hazard to the laboratory. For example, the handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple LAIs.[19] The potential for human cell lines to harbor a bloodborne pathogen led the Occupational Health and Safety Administration (OSHA) to interpret that the occupational exposure to bloodborne pathogens final rule would include human cell lines.[17]

HAZARDOUS CHARACTERISTICS OF LABORATORY PROCEDURES

Investigations of LAIs have identified five principal routes of laboratory transmission. These are parenteral inoculations with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, animal bites and scratches, and inhalation exposures to infectious aerosols. The first four routes of laboratory transmission are easy to detect, but account for less than 20 percent of all reported LAIs. [20] Most reports of such infections do not include information sufficient to identify the route of transmission of infection. Work has shown that the probable sources of infection—animal or ectoparasite, clinical specimen, agent, and aerosol—are apparent in approximately 50 percent of cases.[21]

Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are extremely pervasive, placing the laboratory worker carrying out the procedure and other persons in the laboratory at risk of infection. There is general agreement among biosafety professionals, laboratory directors and principal investigators who have investigated LAIs that an aerosol generated by procedures and operations is the probable source of many LAIs, particularly in cases involving workers whose only known risk factor was that they worked with an agent or in an area where that work was done.

Procedures that impart energy to a microbial suspension will produce aerosols. Procedures and equipment used routinely for handling infectious agents in laboratories, such as pipetting, blenders, non-self contained centrifuges, sonicators and vortex mixers are proven sources of aerosols. These procedures and equipment generate respirable-size particles that remain airborne for protracted periods. When inhaled, these particles are retained in the lungs

creating an exposure hazard for the person performing the operation, coworkers in the laboratory, and a potential hazard for persons occupying adjacent spaces open to air flow from the laboratory. A number of investigators have determined the aerosol output of common laboratory procedures. In addition, investigators have proposed a model for estimating inhalation dosage from a laboratory aerosol source. Parameters that characterize aerosol hazards include an agent's inhalation infective dose, its viability in an aerosol, aerosol concentration, and particle size.[22, 23, 24]

Procedures and equipment that generate respirable size particles also generate larger size droplets that can contain multiple copies of an infectious agent. The larger size droplets settle out of the air rapidly, contaminating the gloved hands and work surface and possibly the mucous membranes of the persons performing the procedure. An evaluation of the release of both respirable particles and droplets from laboratory operations determined that the respirable component is relatively small and does not vary widely; in contrast hand and surface contamination is substantial and varies widely.[25] The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

Technique can significantly impact aerosol output and dose. The worker who is careful and proficient will minimize the generation of aerosols. A careless and hurried worker will substantially increase the aerosol hazard. For example, the hurried worker may operate a sonic homogenizer with maximum aeration whereas the careful worker will consistently operate the device to assuring minimal aeration. Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration.[22] Similar results were shown for pipetting with bubbles and with minimal bubbles. Containment and good laboratory practices also reduce this risk.

POTENTIAL HAZARDS ASSOCIATED WITH WORK PRACTICES, SAFETY EQUIPMENT AND FACILITY SAFEGUARDS

Workers are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous agents. Protection depends on the conscientious and proficient use of good microbiological practices and the correct use of safety equipment. A risk assessment should identify any potential deficiencies in the practices of the laboratory workers. Carelessness is the most serious concern, because it can compromise any safeguards of the laboratory and increase the risk for coworkers. Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for a laboratory staff in order to reduce the inherent risks that attend work with hazardous agents. Not all workers who join a laboratory staff will have these prerequisite traits even though they may possess excellent scientific credentials. Laboratory directors or principal investigators should train and retrain new staff to the point where aseptic techniques and safety precautions become second nature.[26]

There may be hazards that require specialized personal protective equipment in addition to safety glasses, laboratory gowns, and gloves. For example, a procedure that presents a splash hazard may require the use of a mask and a face shield to provide adequate protection. Inadequate training in the proper use of personal protective equipment may reduce its

effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker. For example, a respirator may impart a risk to the wearer independent of the agents being manipulated.

Safety equipment such as Biological Safety Cabinets (BSC), centrifuge safety cups, and sealed rotors are used to provide a high degree of protection for the laboratory worker from exposure to microbial aerosols and droplets. Safety equipment that is not working properly is hazardous, especially when the user is unaware of the malfunction. The containment capability of a BSC is compromised by poor location, room air currents, decreased airflow, leaking filters, raised sashes, crowded work surfaces, and poor user technique. The safety characteristics of modern centrifuges are only effective if the equipment is operated properly. Training in the correct use of equipment, proper procedure, routine inspections and potential malfunctions, and periodic re-certification of equipment, as needed, is essential.

Facility safeguards help prevent the accidental release of an agent from the laboratory. Their use is particularly important at BSL-3 and BSL-4 because the agents assigned to those levels can transmit disease by the inhalation route or can cause life-threatening disease. For example, one facility safeguard is directional airflow. This safeguard helps to prevent aerosol transmission from a laboratory into other areas of the building. Directional airflow is dependent on the operational integrity of the laboratory's heating, ventilation, and air conditioning (HVAC) system. HVAC systems require careful monitoring and periodic maintenance to sustain operational integrity. Loss of directional airflow compromises safe laboratory operation. BSL-4 containment facilities provide more complex safeguards that require significant expertise to design and operate.

Consideration of facility safeguards is an integral part of the risk assessments. A biological safety professional, building and facilities staff, and the IBC should help assess the facility's capability to provide appropriate protection for the planned work, and recommend changes as necessary. Risk assessment may support the need to include additional facility safeguards in the construction of new or renovation of old BSL-3 facilities.

AN APPROACH TO ASSESS RISKS AND SELECT APPROPRIATE SAFEGUARDS

Biological risk assessment is a subjective process requiring consideration of many hazardous characteristics of agents and procedures, with judgments based often on incomplete information. There is no standard approach for conducting a biological risk assessment, but some structure can be helpful in guiding the process. This section describes a five-step approach that gives structure to the risk assessment process.

First, identify agent hazards and perform an initial assessment of risk. Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments.

There are several excellent resources that provide information and guidance for making an initial risk assessment. The BMBL provides agent summary statements for some agents associated with LAIs or are of increased public concern. Agent summary statements also identify

known and suspected routes of transmission of laboratory infection and, when available, information on infective dose, host range, agent stability in the environment, protective immunizations, and attenuated strains of the agent.

A thorough examination of the agent hazards is necessary when the intended use of an agent does not correspond with the general conditions described in the Summary Statement or when an agent summary statement is not available. Although a summary statement for one agent may provide helpful information for assessing the risk of a similar agent, it should not serve as the primary resource for making the risk determination for that agent. Refer to other resources for guidance in identifying the agent hazards.

The *Control of Communicable Diseases Manual* provides information on communicable diseases including concise summaries on severity, mode of transmission, and the susceptibility and resistance of humans to disease.[3] In addition, it is always helpful to seek guidance from colleagues with experience in handling the agent and from biological safety professionals.

Often there is not sufficient information to make an appropriate assessment of risk. For example, the hazard of an unknown agent that may be present in a diagnostic specimen will be unknown until after completing agent identification and typing procedures. It would be prudent in this case to assume the specimen contains an agent presenting the hazardous classification that correlates with BSL-2 unless additional information suggests the presence of an agent of higher risk. Identification of agent hazards associated with newly emergent pathogens also requires judgments based on incomplete information. Consult interim biosafety guidelines prepared by the CDC and the WHO for risk assessment guidance. When assessing the hazards of a newly attenuated pathogen, experimental data should support a judgment that the attenuated pathogen is less hazardous than the wild-type parent pathogen before making any reduction in the containment recommended for that pathogen.

Make a preliminary determination of the biosafety level that best correlates with the initial risk assessment based on the identification and evaluation of the agent hazards. Remember that aerosol and droplet routes of agent transmission also are important considerations in specification of safety equipment and facility design that result in a given BSL level.

Second, identify laboratory procedure hazards. The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

The complexity of a laboratory procedure can also present a hazard. The agent summary statement provides information on the primary laboratory hazards associated with typically routine procedures used in handling an agent. In proposed laboratory procedures where the procedure hazards differ from the general conditions of the agent summary statement or where an agent summary statement is not available, the risk assessment should identify specific hazards associated with the procedures.

Third, make a final determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment. The final selection of the appropriate biosafety level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards described in Chapters 3, 4 and 5 of this publication.

There will be situations where the intended use of an agent requires greater precautions than those described in the agent's Summary Statement. These situations will require the careful selection of additional precautions. An obvious example would be a procedure for exposing animals to experimentally generated infectious aerosols.

It is unlikely that a risk assessment would indicate a need to alter the recommended facility safeguards specified for the selected biosafety level. If this does occur, however, it is important that a biological safety professional validate this judgment independently before augmenting any facility secondary barrier.

It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Preexisting diseases, medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure to infants to certain agents, are some of the conditions that may increase the risk of an individual for acquiring a LAI. Consultation with an occupational physician knowledgeable in infectious diseases is advisable in these circumstances.

Fourth, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment. The protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. In conducting a risk assessment, the laboratory director or principal investigator should ensure that laboratory workers have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of the agent, and have developed good habits that sustain excellence in the performance of those practices. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and BSCs, ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is important insurance that a laboratory worker is capable of working safely.

The laboratory director or principal investigator should also ensure that the necessary safety equipment is available and operating properly. For example, a BSC that is not certified represents a potentially serious hazard to the laboratory worker using it and to others in the laboratory. The director should have all equipment deficiencies corrected before starting work with an agent.

Fifth, review the risk assessment with a biosafety professional, subject matter expert, and the IBC. A review of the risk assessment and selected safeguards by knowledgeable individuals is always beneficial and sometimes required by regulatory or funding agencies, as is the case with the *NIH Guidelines*.[2] Review of potentially high risk protocols by the local IBC should become standard practice. Adopting this step voluntarily will promote the use of safe practices in work with hazardous agents in microbiological and biomedical laboratories.

IN CONCLUSION

Risk assessment is the basis for the safeguards developed by the CDC, the NIH, and the microbiological and biomedical community to protect the health of laboratory workers and the public from the risks associated with the use of hazardous biological agents in laboratories. Experience shows that these established safe practices, equipment, and facility safeguards work.

New knowledge and experiences may justify altering these safeguards. Risk assessment, however, must be the basis for recommended change. Assessments conducted by laboratory directors and principal investigators for the use of emergent agents and the conduct of novel experiments will contribute to our understanding of the risks these endeavors may present and the means for their control. Those risk assessments will likely mirror progress in science and technology and serve as the basis for future revisions of BMBL.

REFERENCES

1. Laboratory biosafety manual. 3rd ed. Geneva: World Health Organization; 2004.
2. NIH guidelines for research involving recombinant DNA molecules. Bethesda: The National Institutes of Health (US), Office of Biotechnology Activities; 2002, April.
3. Heymann DL. Control of communicable diseases manual. 18th ed. Washington, DC: American Public Health Association; 2005.
4. Lennette EH, Koprowski H. Human infection with Venezuelan equine encephalomyelitis virus. JAMA. 1943;123:1088-95.
5. Tigertt WD, Benenson AS, Gochenour WS. Airborne Q fever. Bacteriol Rev. 1961;25:285-93.
6. Centers for Disease Control and Prevention. Fatal Cercopithecine herpesvirus 1 (B virus) infection following a mucocutaneous exposure and interim recommendations for worker protection. MMWR Morb Mortal Wkly Rep. 1998;47:1073-6,1083.
7. Centers for Disease Control and Prevention. Laboratory management of agents associated with hantavirus pulmonary syndrome: interim biosafety guidelines. MMWR Recomm Rep. 1994;43:1-7.
8. Wedum AG, Barkley WE, Helman A. Handling of infectious agents. J Am Vet Med Assoc. 1972;161:1557-67.
9. Jackson RJ, Ramsay AJ, Christensen CD, et al. Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. J Virol. 2001;75:1205-10.
10. Shimono N, Morici L, Casali N, et al. Hypervirulent mutant of *Mycobacterium tuberculosis* resulting from disruption of the mce1 operon. Proc Natl Acad Sci U S A. 2003;100:15918-23.
11. Cunningham ML, Titus RG, Turco SJ, et al. Regulation of differentiation to the infective stage of the protozoan parasite Leishmania major by tetrahydrobiopterin. Science. 2001;292:285-7.
12. Kobasa D, Takada A, Shinya K, et al. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. Nature. 2004;431:703-7.
13. Efstathiou S, Preston CM. Towards an understanding of the molecular basis of herpes simplex virus latency. Virus Res. 2005;111:108-19.

14. Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med. 2005;352:2271-84.
15. Centers for Disease Control and Prevention. Update: Investigation of rabies infections in organ donor and transplant recipients - Alabama, Arkansas, Oklahoma, and Texas. MMWR Morb Mortal Wkly Rep. 2004;53:615-16.
16. Centers for Disease Control and Prevention. Lymphocytic choriomeningitis virus infection in organ transplant recipients--Massachusetts, Rhode Island, 2005. MMWR Morb Mortal Wkly Rep. 2005;54:537-39.
17. Occupational exposure to bloodborne pathogens. Final Rule. Standard interpretations: applicability of 1910.1030 to established human cell lines, 29 C.F.R. Sect. 1910.1030 (1991).
18. Gartner HV, Seidl C, Luckenbach C, et al. Genetic analysis of a sarcoma accidentally transplanted from a patient to a surgeon. N Engl J Med. 1996;335:1494-7.
19. Dykewicz CA, Dato VM, Fisher-Hoch SP, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. JAMA. 1992;267:1349-53.
20. Pike RM. Laboratory-associated infections: incidence, fatalities, causes, and prevention. Annu Rev Microbiol. 1979;33:41-66.
21. Harding AL, Byers KB. Epidemiology of laboratory-associated infections. In: Fleming DO, Hunt DL, editors. Biological safety: principles and practices. 3rd ed. Washington, DC: ASM Press; 2000:35-54.
22. Dimmick RL, Fogl WF, Chatigny MA. Potential for accidental microbial aerosol transmission in the biology laboratory. In: Hellman A, Oxman MN, Pollack R, editors. Biohazards in biological research. Proceedings of a conference held at the Asilomar conference center; 1973 Jan 22-24; Pacific Grove, CA. New York: Cold Spring Harbor Laboratory; 1973. p. 246-66.
23. Kenny MT, Sable FL. Particle size distribution of *Serratia marcescens* aerosols created during common laboratory procedures and simulated laboratory accidents. Appl Microbiol. 1968;16:1146-50.
24. Chatigny MA, Barkley WE, Vogl WF. Aerosol biohazard in microbiological laboratories and how it is affected by air conditioning systems. ASHRAE Transactions. 1974;80(Pt 1):463-469.
25. Chatigny MA, Hatch MT, Wolochow H, et al. Studies on release and survival of biological substances used in recombinant DNA laboratory procedures. National Institutes of Health Recombinant DNA Technical Bulletin. 1979;2.
26. Lennette EH. Panel V common sense in the laboratory: recommendations and priorities. Biohazards in biological research. Proceedings of a conference held at the Asilomar conference center; 1973 Jan 22-24; Pacific Grove, CA. New York: Cold Spring Harbor Laboratory; 1973. p. 353.